

## BEAMLINE

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## PUBLICATION

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## FOR MORE INFORMATION

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## Structural and Biochemical Studies Identify SABP2 as a Methylsalicylate Esterase and Further Implicate it in Plant Innate Immunity

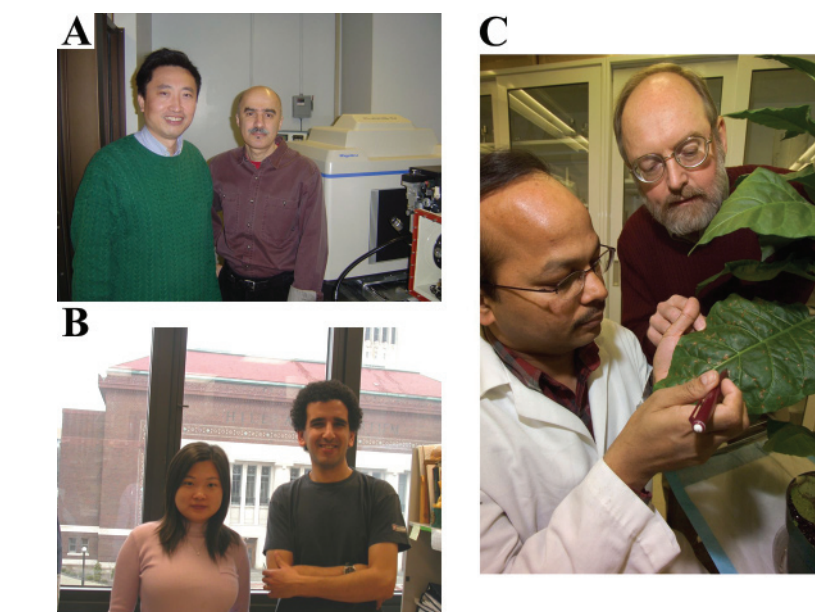
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*Salicylic acid (SA) is a critical signaling compound for the activation of plant defense responses against pathogen infections. SA binding protein 2 (SABP2) displays a high binding affinity for SA and plays a crucial role in the activation of systemic acquired resistance (SAR) — the triggering of defenses in uninfected parts of the plant — to pathogens. As a project of the Northeast Structural Genomics Consortium, we have determined the crystal structures of SABP2, alone and in complex with SA. SA is bound in the active site and is completely shielded from the solvent. Our biochemical studies reveal, for the first time, that SABP2 has strong esterase activity with methylsalicylate (MeSA) as the substrate, and that SA is a potent product-inhibitor of this catalysis. Our results suggest that SABP2 may be required to convert MeSA to SA as part of the signal transduction pathways that activate SAR.*

Salicylic acid (SA) is a critical signaling compound for the activation of plant defense responses both at the site of infection and, systemically, in distal tissues such as leaves. For example, plants that are SA deficient fail to develop systemic acquired resistance (SAR) (the activation of defenses in uninfected parts of the plant), do not express pathogenesis-related (PR) genes in the uninoculated leaves, and display an enhanced susceptibility to pathogens. We recently identified and characterized a high-affinity SA-binding protein (SABP), termed SABP2, from tobacco. Silencing SABP2 expression via RNA interference suppresses the plant's local resistance to Tobacco Mosaic Virus and SA-induced PR-1 gene expression, and blocks the development of SAR.

To understand the biochemical and biological functions of SABP2, we have determined its structure in the absence and presence of SA at up to 2.1 Å resolution. The active site of SABP2 is located at the interface between the "core" and the "cap" domains of the enzyme,



Experimenters on this project: (A) Columbia University: Farhad Forouhar (right) and Yang Chen, (B) University of Michigan: Yue Yang (left) and Eyal Fridman, (C) Boyce Thompson Institute for Plant Research: Dharendra Kumar (left) and Daniel Klessig.

with the "catalytic triad" of amino acids — Ser81, His238, and Asp210 (**Figure 1A**). SA is bound in the active site, where it is completely shielded from the solvent and shows intimate polar and van der

Waals contacts with the enzyme (**Figure 1B**). This provides a molecular explanation for the high affinity of SABP2 for SA.

We next demonstrated that SABP2

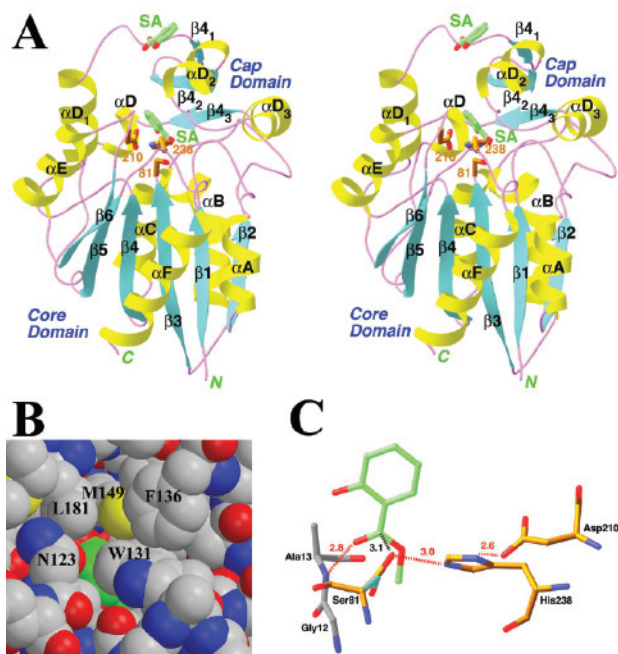
has esterase activity with methylsalicylate (MeSA), a methyl ester of SA, as the substrate (**Figure 2A**) and that SA is a potent product-inhibitor of this activity (**Figure 2B**). While SABP2 displays esterase activity with MeSA and the methylated derivatives of two plant hormones — methyl jasmonate (MeJA) and methyl indole acetic acid (MeIAA) — when they are present at high concentrations, it is highly specific for MeSA (among the substrates tested) at more physiologically relevant concentrations (**Figure 2A**).

To understand how SABP2 can catalyze the hydrolysis of MeSA, we modeled this substrate into the SABP2 active site based on the structure of the complex obtained

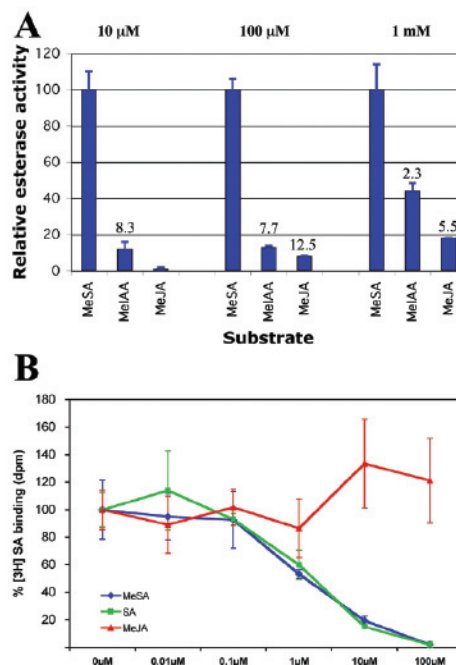
with SA (**Figure 1C**), which is the product of the reaction. By assuming a different torsion angle, the side chain of Ser81 can become hydrogen-bonded to the side chain of His238, completing the catalytic triad (**Figure 1C**). In addition, the side-chain hydroxyl is placed directly over the carboxyl carbon of MeSA, in a perfect position for initiating the nucleophilic attack. The model also shows that the active site is only large enough to accommodate the phenyl ring of MeSA, therefore explaining the poor activity of SABP2 with the MeIAA and MeJA substrates (**Figure 2A**).

These results, together with the genetic and physiological experiments previously reported for SABP2, suggest that MeSA may

have an important role in SAR that is distinct from the role of SA. Since MeSA is more hydrophobic than SA and can thereby cross membranes more readily, it is possible that both short- and long-distance transmission of SA synthesized at the site of infection requires converting it first to MeSA. Our studies suggest that the role of SABP2 in plant host defense may be the hydrolysis of biologically inactive MeSA into active SA in the target cells. The potent inhibition of SABP2 by the product of the reaction, SA, may further help fine-tune intracellular SA levels. The presence of homologous proteins with MeSA esterase activity in other plant species suggests that MeSA may be a component of plant innate immune response in general.



**Figure 1.** Structure of SABP2 in complex with SA. (A). Stereoview of the SABP2 monomer in complex with SA. SA (in green for carbon atoms) is located in the active site. (B). Active site of SABP2 in complex with SA, showing that the SA molecule (in green for carbon atoms) is shielded from the solvent in the active site. (C). Model of the binding mode of the MeSA substrate (green) to SABP2. The side chain of the catalytic Ser81 residue assumes a different conformation for catalysis (cyan and gold in complex with SA and MeSA, respectively). The hydrogen bonds are indicated in dashed lines in red, and the distance between Ser81 and the MeSA carboxylate carbon is indicated in black.



**Figure 2.** Comparisons of methyl esterase activities and binding affinities of SABP2. (A). Relative methyl esterase activity of SABP2 with MeSA, MeIAA, and MeJA substrates at three different concentrations (10  $\mu$ M, 100  $\mu$ M, and 1 mM). The activity with MeSA at each of the substrate concentrations was set at 100%. (B). SABP2 binds MeSA but not MeJA. MeSA (blue) competes with [3H]SA for binding to SABP2 with the same potency as SA (green), whereas MeJA (red) does not compete for binding.